Analysis of Genetic Variation in Upper Willamette River Bull Trout Populations

Draft Report Submitted

March 31, 2009

Final Report Submitted

May 18, 2009

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Summary

Currently there are four local bull trout populations within the upper Willamette River system as defined by the U.S. Fish and Wildlife Service draft bull trout recovery plan: one in the upper McKenzie above Trail Bridge Dam, one in the upper McKenzie below Trail Bridge Dam, one in the South Fork McKenzie River and a re-introduced population in the Middle Fork Willamette River. These four local populations are presently isolated from one another by dams which lack fish passage facilities. Fry have been transferred from Anderson Creek (upper McKenzie below Trailbridge Dam) to other tributaries in the system in order to increase population sizes and re-establish extirpated populations. In this study we used a suite of 16 microsatellite loci to characterize levels of genetic variation within upper Willamette bull trout populations, examine the genetic relationship among bull trout populations, and determine how the relationship among populations had been affected by fry transfers. We also evaluated if levels of genetic variation in a sample of captive-reared bull trout were an adequate representation of the wild source population. We found that levels of genetic variation were lowest in a small population of bull trout located in the upper McKenzie River above Trail Bridge Dam. When compared to other bull trout populations in the mid and lower Columbia River basin, we observed that levels of variation in the Willamette populations tended to be lower than other populations with greater connectivity among spawning areas. Our results suggested the presence of three main populations/groups of bull trout in the upper Willamette: one in Roaring River, one in the upper McKenzie and one group that consisted of Anderson Creek and the tributaries that had received fry from Anderson Creek. Genetic relationships among populations appear to have been affected by contemporary forces including dam construction and fry transfers as well as fidelity to natal spawning tributaries. Levels of genetic variation in the re-introduced population in the Middle Fork Willamette were comparable to those in Anderson Creek, the source population. Estimates of genetic variation were significantly lower in a sample of captive-reared fish compared to the source population in Anderson Creek. Analyses of relatedness suggest that this is because the majority of these fish were closely related (i.e. full and half siblings). Information from this report will form the basis for a genetic management plan

that will provide guidance on what measures can be taken to maintain and perhaps increase current levels of genetic variability in these isolated populations.

Introduction

Bull trout (*Salvelinus confluentus*) are currently listed as a threatened species under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service [USFWS] 1999). Populations have declined in distribution and abundance across the species range due to a number of threats including introductions of nonnative species, habitat degradation, and habitat fragmentation (Rieman et al. 1997). Although the long-term viability of bull trout populations depends on the availability of large connected habitat patches (Rieman and Dunham 2000), in many watersheds bull trout presently exist as a series of isolated populations due to the construction of impassable dams and other barriers (e.g. culverts, irrigation diversion structures) that have fragmented important migratory corridors. The genetic consequences of population isolation and fragmentation have been previously documented for bull trout and include the loss of genetic variability within populations, reduced gene flow among populations, and exclusion of migratory adults from spawning areas (Nerass and Spruell 2001; Costello et al. 2003; Whiteley et al. 2006; DeHaan et al. 2007). Fragmentation of populations by dams and other habitat alterations may also result in genetic bottlenecks (Yamamoto et al. 2004), increased rates of inbreeding (Rieman and Allendorf 2001), and changes in life history (Morita et al. 2000).

The U.S. Fish and Wildlife Service Draft Recovery Plan for bull trout currently recognizes four local populations of bull trout in the upper Willamette River: one population in the upper McKenzie River above Trail Bridge Dam, one population in the upper McKenzie River below Trail Bridge Dam, one population in the South Fork McKenzie River and one population in the Middle Fork (MF) Willamette (Figure 1). In the upper McKenzie River above Trail Bridge Dam bull trout spawn in Sweetwater Creek and the mainstem McKenzie River and below Trail Bridge Dam bull trout spawn in Anderson and Olallie Creeks. In the South Fork McKenzie bull trout spawn in Roaring River and in the MF Willamette bull trout spawn near three springs and adjacent portions of the MF Willamette. Extensive surveys in the MF Willamette in the 1990s documented no bull trout and the population was presumed to be extirpated (USFWS 2002). This population has subsequently been re-introduced using fry from Anderson Creek in the upper McKenzie below Trail Bridge Dam local population (see below).

Bull trout habitat in the upper Willamette River has been extensively fragmented. Although bull trout could migrate among the different spawning areas historically, three of the four local populations (MF Willamette, Roaring River and the McKenzie above Trail Bridge Dam) are presently isolated above mainstem dams which lack upstream fish transport facilities (Figure 1). Additionally, spawning habitat in Sweetwater and Olallie Creeks was inaccessible in the past because of two culverts which blocked upstream migration of adult bull trout in these creeks. These culverts were removed/modified in the 1990s (Sweetwater Creek in 1992, Olallie Creek in 1995) to facilitate fish passage. Extensive habitat fragmentation and the low numbers of spawners in the upper Willamette system raises concerns about the loss of genetic and phenotypic variation through genetic drift and the long term viability of these populations (Rieman and Allendorf 2001).

Given the concerns related to population abundance and habitat fragmentation in the upper Willamette system, a number of management actions have been implemented to help bull trout recovery in the system. In many areas of the upper Willamette where adult bull trout were susceptible to angling, fishing regulations have been modified in order to protect bull trout. In recent years bull trout captured below Trail Bridge Dam and Cougar Dam have been transported upstream of these dams so that they can access spawning areas. As mentioned above, culverts in Olallie and Sweetwater Creeks which limited access to spawning areas were removed or modified to re-establish connectivity. There have also been transfers of bull trout fry among the different spawning areas in the upper Willamette River (Table 1). Following culvert modification in Sweetwater Creek, fry from Anderson Creek were transferred to Sweetwater Creek from 1993 to 1999 in order to help re-establish a spawning population. A relatively small number of fry were also transferred from Anderson to Olallie Creek in 1994 and 1995 to help increase the size of this population following culvert modification. The most extensive fry transfers were from Anderson Creek to the MF Willamette. From 1997 through 2005 over 10,000 fry were transferred to the MF Willamette in an effort to re-establish a spawning population. Starting in 2007 fry collected in Anderson Creek were first taken to the Oregon Department of Fish and Wildlife Leaburg Fish Hatchery for rearing and these fish were transferred to Swift and Bear Creeks in the MF Willamette after six to eight

months of hatchery rearing. An increase in bull trout redd counts in many of the upper Willamette spawning tributaries (Upper Willamette Bull Trout Working Group 2008) suggests that management activities have been successful at increasing population sizes in several of these tributaries (Figure 2).

Although population sizes have increased in many of the upper Willamette spawning tributaries, little information exists regarding levels of genetic variability within these isolated populations, the genetic relationship among remnant isolated populations and how management actions such as transferring fry among spawning tributaries has affected levels of genetic variability in the upper Willamette system. A study by Spruell et al. (2003) using four microsatellite markers found that levels of genetic variation within two upper Willamette bull trout populations were relatively low and that these two populations were most genetically similar to other populations in the lower Columbia River Basin. The development of more variable microsatellite markers and the collection of bull trout from throughout the upper Willamette system seem to warrant a more fine-scale genetic analysis, however. Furthermore, the USFWS Draft Bull Trout Recovery Plan (USFWS 2002), the Upper Willamette Basin Bull Trout Action Plan (Upper Willamette Bull Trout Working Group 2008), and the USFWS 2008 final biological opinion on the operation and maintenance of the U.S. Army Corps of Engineers Willamette Project (USFWS 2008) all identified a fine-scale genetic analysis which could be used to develop a genetic management plan for upper Willamette bull trout as a priority for bull trout recovery planning. The purpose of this project therefore was to conduct a thorough analysis of genetic variability within and among upper Willamette bull trout populations. Our project had four specific objectives:

1) To determine the level of genetic variability within the upper Willamette spawning populations and examine how habitat fragmentation has affected levels of variability
2) To examine the genetic relationship among bull trout spawning populations and determine how transfers of fry have affected these relationships
3) To determine if levels of genetic variation observed in fry that have been reared at the Oregon Department of Fish and Wildlife's Leaburg Hatchery are

representative of the source population in Anderson Creek and to determine if Anderson Creek fry are a suitable population for fry translocations

4) To use the baseline genetic dataset developed under objectives 1 and 2 to assign adults collected outside spawning areas to their most likely population of origin

Methods

Sample Collection

Baseline sampling efforts utilized a number of different techniques and targeted various life history stages in each of the different spawning tributaries. Collection efforts in Anderson Creek, the upper McKenzie River, Olallie Creek, and Sweetwater Creek targeted juvenile bull trout. Juveniles from Anderson Creek were collected in a screw trap downstream from the Highway 126 culvert, juveniles in the upper McKenzie River were collected with dip nets and juveniles in Olallie and Sweetwater Creeks were collected in minnow traps. Baseline collection efforts in the MF Willamette targeted both juveniles and adults and fish were collected in a screw trap upstream from the Forest Road 2143 bridge. Baseline collection efforts in Roaring River targeted adult fish collected in a screw trap. Fin clips were taken from all individuals collected and stored in 100% non-denatured ethanol at ambient temperatures.

A number of adult bull trout were collected outside of the spawning tributaries and were treated as unknown origin fish for population assignments. These fish were collected below Trail Bridge Dam by angling (n = 9) and in Trail Bridge Reservoir (n = 42) using trap nets and frame traps. We also obtained fin clips from a number of bull trout fry that were taken to the Leaburg Hatchery in 2007 and died prior to their release. These fish were originally collected in Anderson Creek in 2007 and were to be released into the MF Willamette. These captive-reared samples were analyzed for comparison with the age 1 and older juveniles collected in Anderson Creek .

Laboratory Analysis

DNA was extracted from all samples using a modified chelex extraction protocol (Miller and Kapuscinski 1996). All individuals were genotyped at a suite of 16

microsatellite loci; *Omm1128, Omm1130* (Rexroad et al. 2001), *Sco102, Sco105, Sco106, Sco107, Sco109,* (Washington Dept. of Fish and Wildlife *unpublished*), *Sco200, Sco202, Sco212, Sco215, Sco216, Sco218, Sco220* (DeHaan and Ardren 2005), *Sfo18* (Angers et al. 1995) and *Smm22* (Crane et al. 2004). PCR reactions were carried out in 10µl volumes containing 2µl of template DNA, 5µl of 2X QIAGEN Multiplex PCR Master Mix (final concentration of 3mM $MgCl_2$), and 0.2µl of oligonucleotide PCR primer mix. Primer mix concentrations and annealing temperatures for each multiplex are given in Appendix 1. PCR conditions were as follows: initial denaturation at 95°C for 15 minutes, then 29 cycles of 95°C for 30 seconds, 90 seconds at the multiplex specific annealing temperature and 60 seconds primer extension at 72°C, followed by a final extension at 60°C for 20 minutes. Following PCR, capillary electrophoresis was carried out on an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) following the manufacturer's protocols. The G5 filter set was used to produce electropherograms, and electrophoresis data was analyzed using the program Genemapper v4.0 (Applied Biosystems Inc.).

Statistical Analysis

For statistical analysis, bull trout were grouped according to the six spawning tributaries they were collected from: Anderson Creek, Olallie Creek, Sweetwater Creek, Upper McKenzie (Carmen Bypass Reach), Roaring River, and MF Willamette. Captive-reared samples from Leaburg Hatchery were grouped separately so that we could make comparisons between the captive-reared fish and Anderson Creek. Collections from the six spawning tributaries and the captive-reared individuals were tested for conformance to Hardy-Weinberg equilibrium (HWE) using the program GENEPOP v3.4 (Raymond and Rousset 1995). GENEPOP was also used to test each population for linkage disequilibrium. Tests for HWE and linkage disequilibrium provide a means to evaluate if a random sample has been collected from a population or tributary. Significance values for HWE and linkage disequilibrium tests were adjusted for multiple comparisons using a sequential Bonferroni adjustment (Rice 1989). We used the program GDA (Lewis and Zaykin 2001) to estimate levels of genetic variation including mean numbers of alleles per locus and observed and expected heterozygosity within each population. In addition

we used the program HP-Rare v1.0 (Kalinowski 2005) to estimate allelic richness for each population based on a minimum sample size of 40 genes (two times the minimum sample size). This program provides estimates of allelic richness that have been corrected for differences in sample size between populations. Populations were also tested for evidence of recent (within the past 4-6 generations) genetic bottlenecks using the program BOTTLENECK (Cornuet and Luikart 1996) assuming a two-phased model of mutation. This method tests for an excess of heterozygotes relative to the frequency of alleles in the population (Luikart and Cornuet 1998).

We used the program Fstat v2.9.3.2 (Goudet 2001) to estimate the overall level of genetic variation among spawning populations (F_{ST}; Weir and Cockerham 1984) and the associated 95% confidence level based on 1000 bootstrap replicates. The sample of captive-reared fish was also included in this analysis. Fstat was also used to estimate levels of genetic variation (F_{ST}) among all population pairs and to test pairwise estimates for significance. A Bonferroni correction (Rice 1989) was used to adjust significance values of pairwise F_{ST} estimates for multiple comparisons. Using GENEPOP, we performed a chi-squared contingency analysis to determine if there were significant differences in allele frequencies among the seven populations (i.e. genetically distinct spawning groups). P-values were adjusted for multiple comparisons using a Bonferroni correction (Rice 1989) as well as the B-Y FDR correction described in Narum (2006).

We used two methods to examine the spatial genetic relationship among populations. We first examined the multi-dimensional genetic relationship among populations by performing a correspondence analysis (FCA) using the program GENETIX (Belkhir et al. 2004). This method is similar to a principal component analysis and provides an unbiased graphical approach for viewing the data where individuals that are more genetically similar cluster together on the graph. We also generated a consensus neighbor-joining tree using the program Phylip v3.6 (Felsenstein 1993). The bootstrap procedure was first used to generate 1,000 replicate datasets based on our observed allele frequencies. We then estimated Cavalli-Sforza and Edwards (1967) chord distances between all population pairs and generated a consensus neighbor-joining tree based on these values.

We also wished to compare levels of genetic variation observed in the captive-reared individuals to those observed in the source population in Anderson Creek to determine if the level of variation observed in the source population was adequately represented in the fish transported to the hatchery. We performed Wilcoxon ranked sum tests to determine if there was a significant difference in measures of genetic diversity including expected and observed heterozygosity and allelic richness between the captive-reared fish and the juveniles collected from Anderson Creek. We used the program Kinship v1.3.1 (Goodnight and Queller 1999) to estimate the degree of relatedness (Rxy; Queller and Goodnight 1989) among all pairs of individuals in each group. This measure provides an estimate of how much genetic material two individuals have in common relative to the population average. In this case, pairwise relatedness values were compared to the population average observed in the juvenile samples from Anderson Creek, the wild source population. Estimates of Rxy range from -1.0 to 1.0 with a value of 0 suggesting two individuals are unrelated, positive values indicating increased relatedness and negative values indicating two individuals are less related than the population average. We performed a Wilcoxon test to determine if there was a significant difference in the distribution of relatedness values between the samples. Additionally we used the methods of Wang (2004) implemented in the program COLONY to partition the Anderson Creek juveniles and the captive-reared fish into half and full sibling families. We examined levels of genetic variation among the Anderson Creek juveniles and the captive-reared fish (F_{ST}) to determine if there was significant genetic divergence between these two populations and if so, how this compared to the level of variation we observed among other population pairs in the upper Willamette system.

In order to assess our ability to correctly assign unknown fish to their population of origin we performed a jackknife analysis of our baseline dataset using the program WhichRun v4.1 (Banks and Eichert 2000). With this procedure each individual fish is removed from the baseline dataset and treated as an unknown. The allele frequencies for each population are then recalculated without that individual, and the individual is assigned to its most likely population of origin based on a maximum likelihood algorithm. The number of individuals that are assigned to their true population of origin provides a means of estimating the statistical power of the baseline dataset to assign

unknown individuals. Once we had determined the ability of the baseline dataset to assign individuals, we used WhichRun to assign adults collected in Trail Bridge Reservoir and below Trail Bridge Dam to their first and second most likely population of origin. Confidence estimates for our assignments represent the likelihood ratio between the first and second most likely populations (i.e. likelihood individual originated from population #1/likelihood individual originated from population #2).

Results

Levels of genetic variation within populations

Four of the 16 microsatellite loci we analyzed, *Sco102*, *Sco202*, *Sco215* and *Sfo18*, were fixed for one allele (i.e. there was no variation at these loci). All six populations conformed to HWE expectations with the exception of the upper McKenzie population (Carmen Bypass Reach) which deviated from HWE at a single locus (*Omm1128*) due to a deficiency of heterozygotes. The sample of captive-reared fish deviated from HWE at three loci: *Omm1130* and *Sco109* due to a heterozygote excess and *Sco220* due to a heterozygote deficiency. All pairs of loci were in linkage equilibrium in the samples from MF Willamette and Sweetwater Creek. The remaining populations had the following number of locus pairs (out of 66) that were out of equilibrium: Anderson Creek 12 pairs of loci, upper McKenzie 2 pairs of loci, Olallie Creek 3 pairs of loci, Roaring River 4 pairs of loci, and the captive-reared fish had 8 pairs of loci out of equilibrium.

All estimates of genetic variation were lowest in the samples collected from the upper McKenzie River (Table 2). Estimates of the mean number of alleles per locus, allelic richness, and expected heterozygosity were all greatest in the juvenile samples from Anderson Creek (4.813, 4.240, and 0.488 respectively; Table 2). Observed heterozygosity was greatest in the samples from Sweetwater Creek (0.522; Table 2). None of the populations showed evidence of a recent genetic bottleneck.

Levels of genetic variation among populations

The overall level of genetic variation among populations (i.e. F_{ST}) we observed was 0.181 and was found to be significantly different from 0.0 (95% C.I. = 0.143-0.225).

We observed the least amount of pairwise genetic variation among the populations in Sweetwater Creek and Anderson Creek and the MF Willamette and Anderson Creek (both estimates of pairwise F_{ST} = 0.021; Table 3) and the greatest amount of genetic variation among the upper McKenzie population and Roaring River (pairwise F_{ST} = 0.346; Table 3). In general we observed lower estimates of variation among Anderson Creek and populations that had received fry transfers from Anderson Creek (e.g. MF Willamette, Sweetwater) and the level of variation among remnant populations (Anderson, Roaring River, upper McKenzie) was considerably greater (Table 3). All pairwise estimates of variation were significantly different from 0.0 following Bonferroni correction. Chi-squared contingency tests revealed that allele frequencies were significantly different among all population pairs following Bonferroni and B-Y FDR correction.

Axis 1 on the FCA plot separated the individuals from Roaring River (dark blue squares) from all other populations and explained 11.27% of the variance we observed. Axis 2 on the FCA plot separated the majority of the upper McKenzie samples (white squares) from the other samples and explained 5.63% of the variation (Figure 3). The remaining samples from Anderson Creek, Olallie Creek, Sweetwater Creek, MF Willamette and the captive-reared fish all clustered together on the plot (Figure 3). A third axis on the graph (not shown) showed some clustering of individuals from the remaining populations. Anderson Creek, the fish reared at the hatchery, and the populations that had received fry transfers from Anderson Creek all grouped together on the neighbor-joining tree with low bootstrap support (Figure 4). Branches on the tree for these populations were relatively short indicating increased genetic similarity. The samples from Roaring River and the upper McKenzie grouped separately from this cluster with 100% bootstrap support (Figure 4).

Comparisons between captive-reared fish and Anderson Creek

As noted above we observed three loci that deviated from HWE expectations in the captive-reared fish and none in the Anderson Creek juveniles. Estimates of genetic variation within the captive-reared sample of fish were lower than those observed in Anderson Creek (Table 2). Wilcoxon signed rank tests indicated that there was a

significant difference in allelic richness (P = 0.003) and expected heterozygosity (P = 0.014) between the two samples but no significant difference in observed heterozygosity (P = 0.485). Both pairwise estimates of F_{ST} (F_{ST} = 0.047) and chi-squared contingency analysis indicated that there was a significant difference in allele frequencies among the two groups.

The mean level of pairwise relatedness for the Anderson Creek juveniles was 0.040 and the mean level of pairwise relatedness for the captive-reared fish was 0.104. A Wilcoxon test indicated there was a significant difference in the distribution of relatedness values between the two groups (P<0.0001; Figure 5). Using the program COLONY we identified 24 full sibling families and 12 half sibling families in the sample of Anderson Creek juveniles. All of the full sibling families contained four or less individuals with the exception of one family of 10 individuals. In the group of captive-reared fish we identified 12 full sibling families and seven half sibling families. Ten of the full sibling families contained three or less individuals. The remaining two full sibling families contained seven and 23 individuals and these two families shared one parent (i.e. half sibling families). In other words, 30 of the 43 captive-reared fish we analyzed shared one parent.

Genetic Assignments

The proportion of individuals correctly assigned to their population of origin in the jackknife analysis ranged from 0.680 for Anderson Creek to 1.000 for Roaring River (Table 4). None of the individuals from the MF Willamette were assigned to the MF Willamette; instead they all assigned to either Anderson Creek or one of the populations that had received fry from Anderson Creek. Of the nine fish collected below Trail Bridge Dam, two were genetically assigned to Anderson Creek, five were assigned to the upper McKenzie and two were assigned to Sweetwater Creek. Of the 42 fish collected in Trail Bridge Reservoir, one was assigned to Anderson Creek, 32 were assigned to the upper McKenzie and nine were assigned to Sweetwater Creek.

Discussion

Levels of variation within and among populations

Maintaining genetic variation is important for the long term persistence of natural populations (Allendorf and Luikart 2007). Among the spawning tributaries we examined in this study, levels of genetic variation were considerably lower in the upper McKenzie River (Table 2) suggesting that this tributary supports a spawning population with a relatively small effective size. Although redd counts in the upper McKenzie have increased in recent years (Figure 2), they remain relatively low suggesting a small spawning population persists in this tributary (Upper Willamette Bull Trout Working Group 2008). Although none of the populations showed evidence of a recent genetic bottleneck (approximately the last 5 generations), low levels of genetic variation and low population size suggest that a bottleneck likely occurred in this population historically. Given the correlation between reductions in genetic variability and increased risk of extinction, the remnant population in the upper McKenzie seems to be a high priority for conservation measures to help to increase the size of this population.

Recently the USFWS completed an analysis of genetic variation across the geographic range of bull trout in the United States. Our study of Willamette bull trout utilized the same set of same genetic markers as this range-wide study which allows us to compare levels of genetic variation observed in the Willamette to other populations in the Columbia River basin and across the species range. Estimates of genetic variation including allelic richness and observed and expected heterozygosity ranged widely among lower and mid-Columbia bull trout populations and estimates from Willamette populations were close to the mean values we observed (USFWS *unpublished data*). The upper McKenzie population was an exception however; levels of variation in the upper McKenzie were the lowest we observed among all the lower and mid Columbia populations. Previous studies have demonstrated that populations of fish isolated above barriers often show reduced levels of genetic variability (Costello et al. 2003; Wofford et al. 2005; Neville et al. 2006; DeHaan et al. 2007). When compared with other watersheds with greater connectivity among bull trout populations, levels of variation within the Willamette system were much lower. For example, in the Metolius River, where no barriers to migration exist, we observed greater mean estimates of allelic richness, expected and observed heterozygosity (5.65, 0.58 and 0.59 respectively; DeHaan et al.

2008). These results suggest that isolation has contributed to reductions in levels of genetic variation within upper Willamette bull trout populations.

The relatively high level of genetic variation we observed among populations (global F_{ST} = 0.181) is similar to that observed in other bull trout populations across the species range and suggests a high degree of genetic population structure (Nerass and Spruell 2001; Costello et al. 2003; Whiteley et al. 2006; DeHaan et al. 2007). The FCA plot (Figure 3) and the neighbor-joining tree (Figure 4) suggest that there are three genetically distinct groups of bull trout in the upper Willamette; one in the upper McKenzie, one in Roaring River and one group consisting of Anderson Creek and the tributaries that have received fry transfers from Anderson Creek. Contemporary forces including dam construction have played a large part in shaping the genetic population structure of upper Willamette bull trout populations. Small isolated populations such as those in the upper Willamette experience increased rates of genetic drift and subsequently differences among populations become accentuated (Allendorf and Luikart 2007). The fact that multiple loci which are variable in other bull trout populations were fixed in this study suggests increased rates of genetic drift in the upper Willamette and this likely contributes to the relatively high levels of variation we observed among the different populations. Fry transfers among populations have also influenced the genetic structure of upper Willamette bull trout. All of the populations that have received fry from Anderson Creek grouped together on the FCA plot and the NJ tree. Furthermore pairwise F_{ST} estimates among these populations were substantially lower than estimates among populations that had not received fry. Although bull trout populations in close geographic proximity tend to be more genetically similar (Nerass and Spruell 2001; Costello et al. 2003; Whiteley et al. 2006) the upper McKenzie River is geographically close to Anderson Creek and the populations that received fry (except MF Willamette), yet it is genetically quite diverged (Figure 4; Table 3).

Despite the influence that contemporary factors have had on the genetic structure of bull trout in the upper Willamette, historical processes have likely also played a role in shaping patterns of population structure. Data suggest that although individuals could migrate throughout the upper Willamette River historically, there was likely a high level of fidelity to spawning tributaries and genetically distinct spawning populations existed

within tributaries. The Roaring River population provides evidence of this; many of the most common alleles observed in the Roaring River population at several loci are not found in Anderson Creek or the upper McKenzie. If spawning individuals frequently migrated among these populations in the past we would not expect to see as many private alleles in this remnant population. Patterns of bull trout population structure have been linked to historical processes in many other watersheds as well (Costello et al. 2003; Whiteley et al. 2006).

The MF Willamette appears most genetically similar to Anderson Creek, despite the fact that it is separated from Anderson Creek by several hundred river kilometers and multiple impassable dams. The population in the MF Willamette was presumed extirpated and was re-introduced using fry from Anderson Creek starting in the late 1990s. Spawning adults and juveniles observed in the MF Willamette following re-introduction efforts were presumed to have originated in Anderson Creek but the possibility existed that some of these fish were remnant Middle Fork fish. The fact that we observed no loci that deviated from HWE or linkage equilibrium in the MF Willamette collection suggests a single spawning population derived from Anderson Creek is present in the MF Willamette. One important question is whether the re-introduced population in the MF Willamette is an adequate representation of the genetic variation present in Anderson Creek. We observed no major differences in measures of allelic richness, expected and observed heterozygosity between these two populations suggesting that levels of genetic variation within the MF Willamette were comparable to those observed in Anderson Creek.

Comparisons between Anderson Creek and captive-reared fish

Estimates of genetic variation including allelic richness and expected heterozygosity were significantly lower in the group of captive-reared fish than the juveniles collected in Anderson Creek (the source population). We also observed that three of the 12 variable loci in the group of captive-reared fish deviated from HWE. Two of these deviations were due to a deficiency of heterozygotes, often an indication that a number of closely related individuals have been sampled. Analyses of the degree of relatedness among the captive-reared fish indicated that the majority of these fish (30 of

43) were from a half sibling family composed of two large full-sibling families. The large number of related individuals in the group of fish that were reared in the hatchery could be a cause for concern. Releasing large numbers of closely related captive/hatchery-reared fish into a wild population can lead to a reduction in effective population size in the wild population (Ryman and Laikre 1991) as well as an increased risk of inbreeding (Duchesne and Bernatchez 2002).

There are several possible explanations for the increased number of related individuals we observed in the sample of captive-reared fish. One explanation is that certain family groups were more likely to migrate downstream as fry rather than juveniles. The fact that we did not see similar decreases in genetic variation and increased relatedness in populations that received fry from Anderson (e.g. MF Willamette, Sweetwater Creek) does not seem to support this hypothesis however. It is important to note though, that samples from other populations represent the offspring from multiple years' worth of fry transfers whereas the captive-reared sample consisted of a single year class. Alternatively, the time period over which fish were collected for hatchery rearing in 2007 may have led to the collection of a group of related fish. Although fry migrated downstream in Anderson Creek from February through August, the fish that were reared in the hatchery were collected over a span of three weeks (Figure 6). Previous studies have demonstrated that in some salmonid species, young-of-the year and juveniles are often distributed in family groups within streams and during downstream migration (Carlsson et al. 2004; Olsen et al. 2004). If bull trout also migrate downstream in family groups, selecting fish from a truncated portion of the total downstream migration period could lead to the collection of several related individuals. Increased relatedness among the captive-reared fish may also be due to the method in which the fish were sampled at the hatchery; genetic samples were taken from hatchery mortalities only. If certain family groups had greater mortality rates in the hatchery, this could explain the large number of related fish in the group we analyzed. In order to test this hypothesis we would need to compare the hatchery mortalities to the fish that survived in the hatchery, unfortunately we do not have genetic samples from captive-reared fish that survived and were released into the MF Willamette in 2007.

In 2008 bull trout fry collected in Anderson Creek and transported to the hatchery were collected throughout a greater portion of the downstream migration period. Genetic samples have been collected from the majority of these fish but they were not included in the current study. Analysis of these samples in the future could help to clarify whether increased relatedness we observed among the 2007 captive-reared fish was due to the fact that only certain families migrate downstream as fry, if the sample of fry taken to the hatchery in 2007 was biased due to the short collection period, if the sample of fish selected for genetic analysis (mortalities only) was biased towards a small number of family groups or if the increase in relatedness we observed was due to a combination of these factors. We hope to be able to include this information in the genetic management plan.

Genetic population assignments

Results of the jackknife analysis showed that we had a high degree of confidence for assigning unknown bull trout to their correct population or genetic group (i.e. Anderson Creek and associated tributaries). The number of baseline samples correctly assigned from the upper McKenzie and Roaring River was greater than 0.90. Although assignment success was lower for the other populations, individuals in populations that had received fry from Anderson Creek were nearly all assigned to Anderson or another population that had received Anderson fry. Genetic population assignments can be useful in the future for assigning unknown origin fish collected downstream of dams or in the mainstem McKenzie and Willamette to their most likely population of origin. The fact that five of nine fish collected below Trail Bridge Dam assigned to the upper McKenzie population provides evidence that bull trout in the Willamette are moving downstream through dams and potentially being excluded from natal spawning populations. The exclusion of large migratory bull trout from local spawning populations has been previously recognized as threat to bull trout persistence, particularly in small populations such as the upper McKenzie (Rieman et al. 1997; Rieman and Dunham 2000; Nerass and Spruell 2001).

Genetic Management Plan

Our results indicate that isolation of bull trout populations in the upper Willamette River has contributed to reduced genetic variability within populations. In the Willamette River, connectivity among isolated populations has been supplemented by transfers of fry from Anderson Creek. Although these methods appear to have been effective at both re-establishing and increasing the size of populations, questions regarding the genetic implications of these measures persist. For example, now that a population has been re-established in the MF Willamette, is it necessary to continue to transfer fish from Anderson Creek to this tributary in order to maintain levels of genetic variation? If so, how many fish are necessary each year/generation? Previous studies have suggested that one migrant per generation may be enough to maintain levels of genetic variation within a population; however, this may not be adequate in the case of small isolated populations (Mills and Allendorf 1996). Furthermore, demographic, behavioral and environmental data may be equally important when making decisions regarding translocations (Tallmon et al. 2004; Hedrick 2005).

Information presented in this report regarding levels of variation within each population and current levels of gene flow among populations will form the basis for a genetic management plan that provides guidance on what measures can be taken to maintain and perhaps increase current levels of genetic variability. We plan to explore a number of different management strategies in this plan and discuss the pros and cons associated with different strategies. Specifically we hope to address: which populations should serve as donor/source populations for future translocation efforts, which populations should be prioritized for receiving fry in the future, and how many successful migrants or translocated fry per year or generation are necessary for maintaining and increasing levels of diversity. We would also like to provide further analysis of the captive-reared fish including genetic analysis of subsequent sampling years (2008 and possibly 2009) that were representative of the entire fry migration period. Additional analyses will allow us to identify collection and release strategies that help to maximize effective size and genetic variation in the captive-reared fish. Funding for the development of a genetic management plan has been provided to Abernathy Fish Technology Center by the U.S. Army Corps of Engineers and we anticipate completion of a draft genetic management plan for review by September 30, 2009.

Acknowledgements

Funding for this project was provided by the U.S. Army Corps of Engineers Portland District Office. Samples collected for this project were provided by the U.S. Forest Service, Oregon Department of Fish and Wildlife and Stillwater Sciences. Denise Hawkins provided assistance with data analysis and interpretation and Patty Crandell provided comments on a draft version of this report. We would especially like to thank the Upper Willamette Bull Trout Working Group for their enthusiasm for establishing this project, providing background information related to Willamette bull trout populations and their help interpreting the results presented in this report. The findings and conclusions in this report are those of the author and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

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Table 1. Numbers of bull trout fry transferred from Anderson Creek to Willamette River spawning tributaries from 1993 to 2005. Data courtesy of U.S. Forest Service and Oregon Department of Fish and Wildlife.

Year	Tributary		
	Olallie Cr.	Sweetwater Cr.	MF Willamette R.
1993	-	314	-
1994	245	507	-
1995	313	590	-
1996	-	894	-
1997	112	1193	202
1998	-	1889	1497
1999	-	997	1978
2000	-	-	2787
2001	-	-	1458
2002	-	-	290
2003	-	-	1462
2004	-	-	617
2005	-	-	142
TOTAL	**670**	**6384**	**10433**

Table 2. Estimates of genetic variation (based on 12 variable microsatellite loci) within six upper Willamette bull trout spawning populations as well as Anderson Creek fry reared at Leaburg Fish Hatchery.

Population	**n**	**A**	**A_R**	**H_e**	**H_o**
Captive-Reared	43	3.938	3.545	0.443	0.481
Anderson Creek	50	4.813	4.240	0.488	0.507
Upper McKenzie	25	2.813	2.706	0.345	0.321
Olallie Creek	39	4.250	3.755	0.417	0.412
Sweetwater Creek	20	3.938	3.938	0.482	0.522
MF Willamette	26	4.375	4.218	0.468	0.450
Roaring River	84	3.750	3.341	0.417	0.433
Mean		**3.982**	**3.678**	**0.437**	**0.446**

n = # Samples analyzed
A = Mean # alleles per locus
A_R = Allelic richness
H_e = Heterozygosity expected
H_o = Heterozygosity observed

Table 3. Estimates of genetic variation (F_{ST}) among six upper Willamette River bull trout populations and fish reared at Leaburg Fish Hatchery.

	Captive-Reared	Anderson Creek	Upper McKenzie	Olallie Creek	Sweetwater Creek	MF Willamette
Anderson Creek	0.047					
Upper McKenzie	0.221	0.169				
Olallie Creek	0.097	0.056	0.252			
Sweetwater Creek	0.055	0.021	0.232	0.079		
MF Willamette	0.042	0.021	0.163	0.069	0.042	
Roaring River	0.267	0.219	0.346	0.250	0.243	0.219

Table 4. Proportion of individuals assigned to each baseline population in the jackknife analysis.

Collected From:	Assigned To: Anderson Creek	Upper McKenzie	Olallie Creek	Sweetwater Creek	MF Willamette	Roaring River
Anderson Creek	**0.680**	0.060	0.100	0.160	0.000	0.000
Upper McKenzie	0.000	**0.920**	0.000	0.080	0.000	0.000
Olallie Creek	0.077	0.000	**0.846**	0.077	0.000	0.000
Sweetwater Creek	0.200	0.000	0.000	**0.800**	0.000	0.000
MF Willamette	0.500	0.115	0.077	0.308	**0.000**	0.000
Roaring River	0.000	0.000	0.000	0.000	0.000	**1.000**

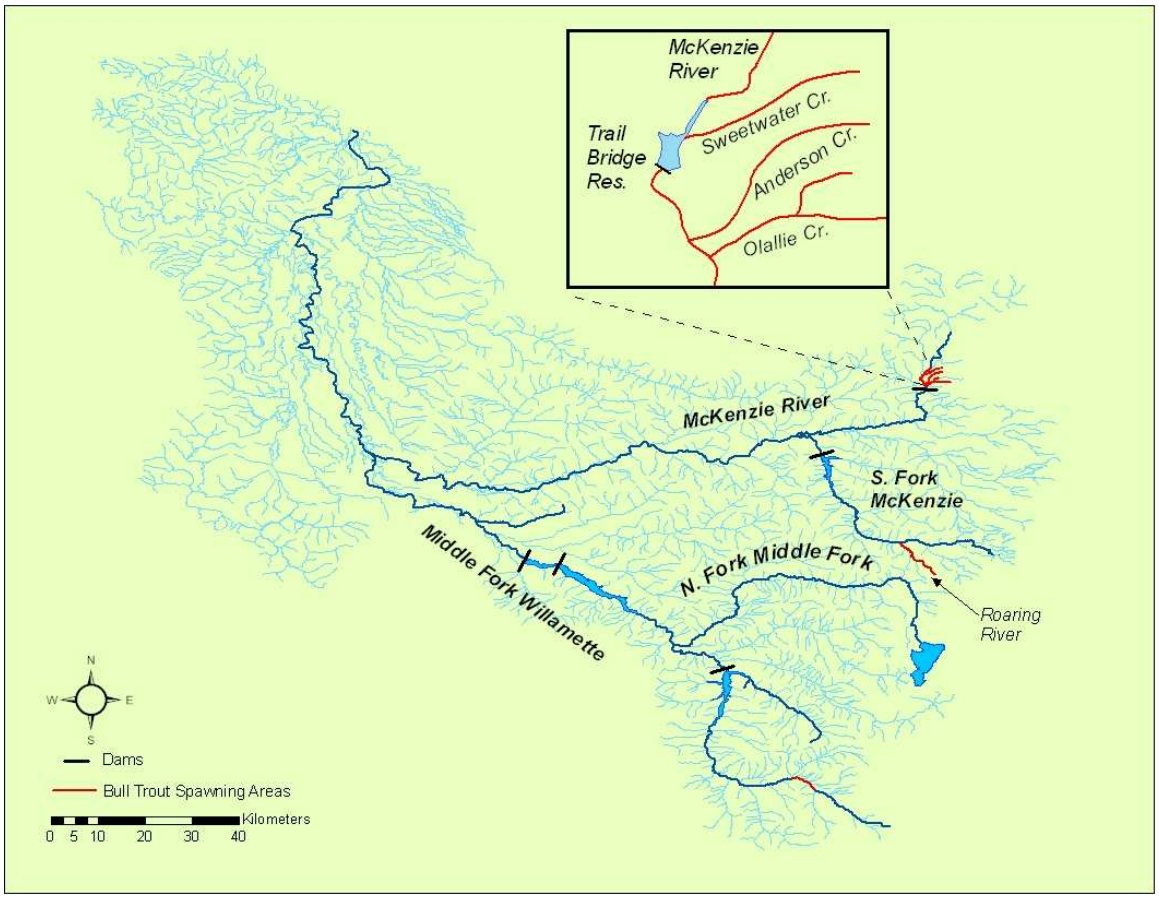

Figure 1. Upper Willamette River system, Oregon. Bull trout spawning areas (sampling locations for this study) are shown in red.

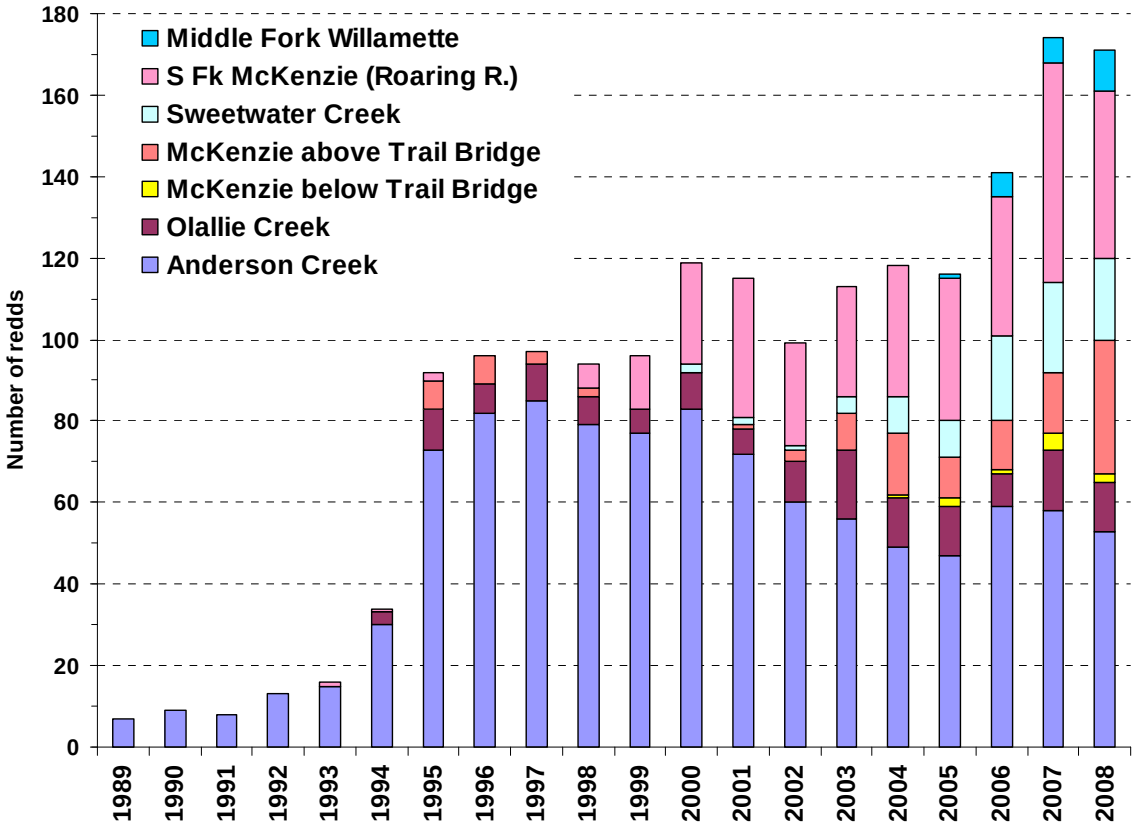

Figure 2. Total number of bull trout redds counted annually in the Upper Willamette River core area since counts began in 1989. Data for redd counts prior to 1999 should be interpreted with caution, as some surveys only included a portion of the known spawning distribution (as discovered in subsequent surveys) and were not repeated intra-annually with the increased frequency of later surveys. Data provided by the Oregon Department of Fish and Wildlife and the U.S. Forest Service, Willamette National Forest.

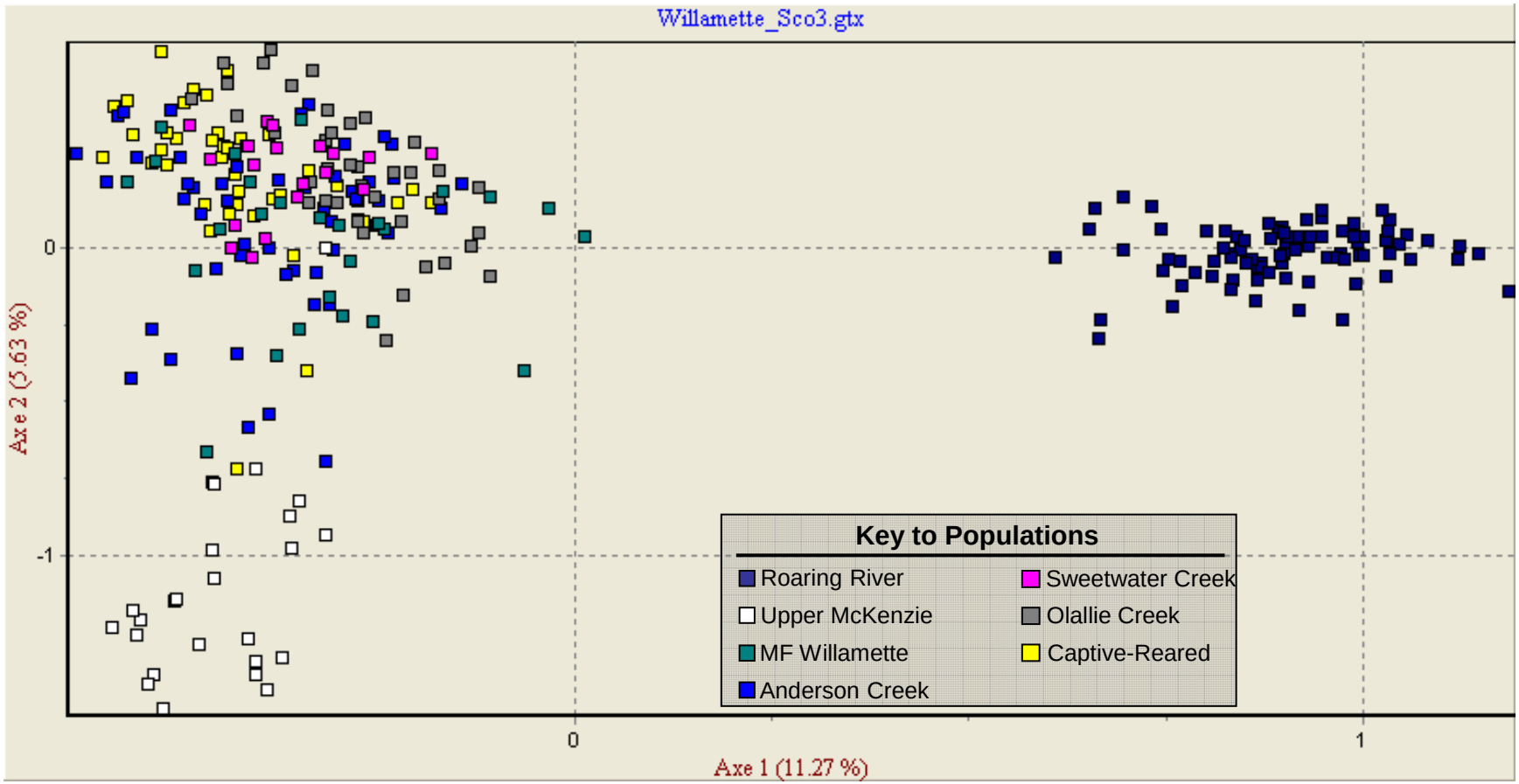

Figure 3. Correspondence analysis (FCA) of bull trout collected in the upper Willamette River system. Each point on the graph represents an individual bull trout in the analysis. Points that cluster closer together are more genetically similar.

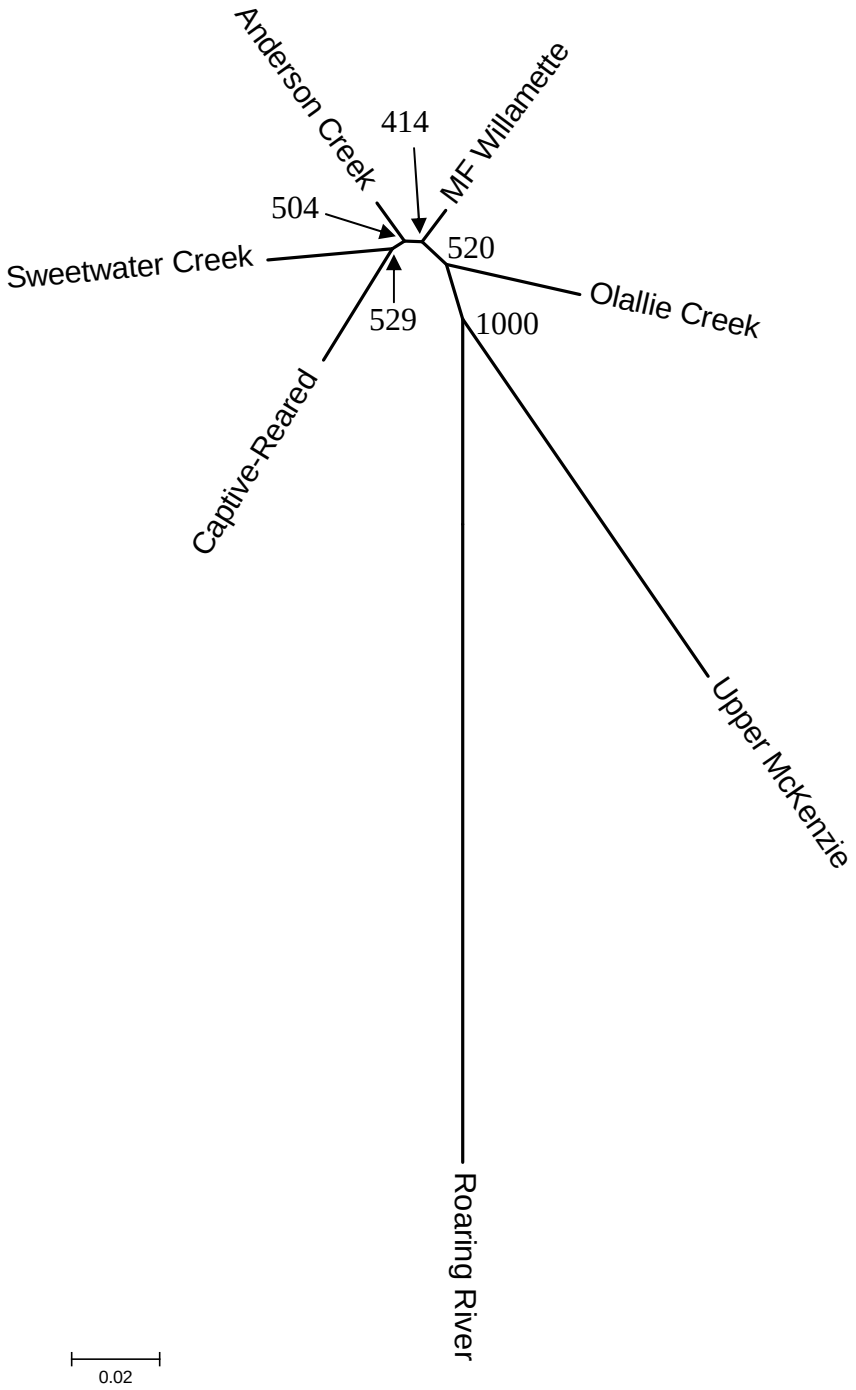

Figure 4. Neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance showing the genetic relationship among the seven sampling sites in this study. Values on the nodes represent the number of bootstrap replicates (out of 1000) that showed the displayed arrangement.

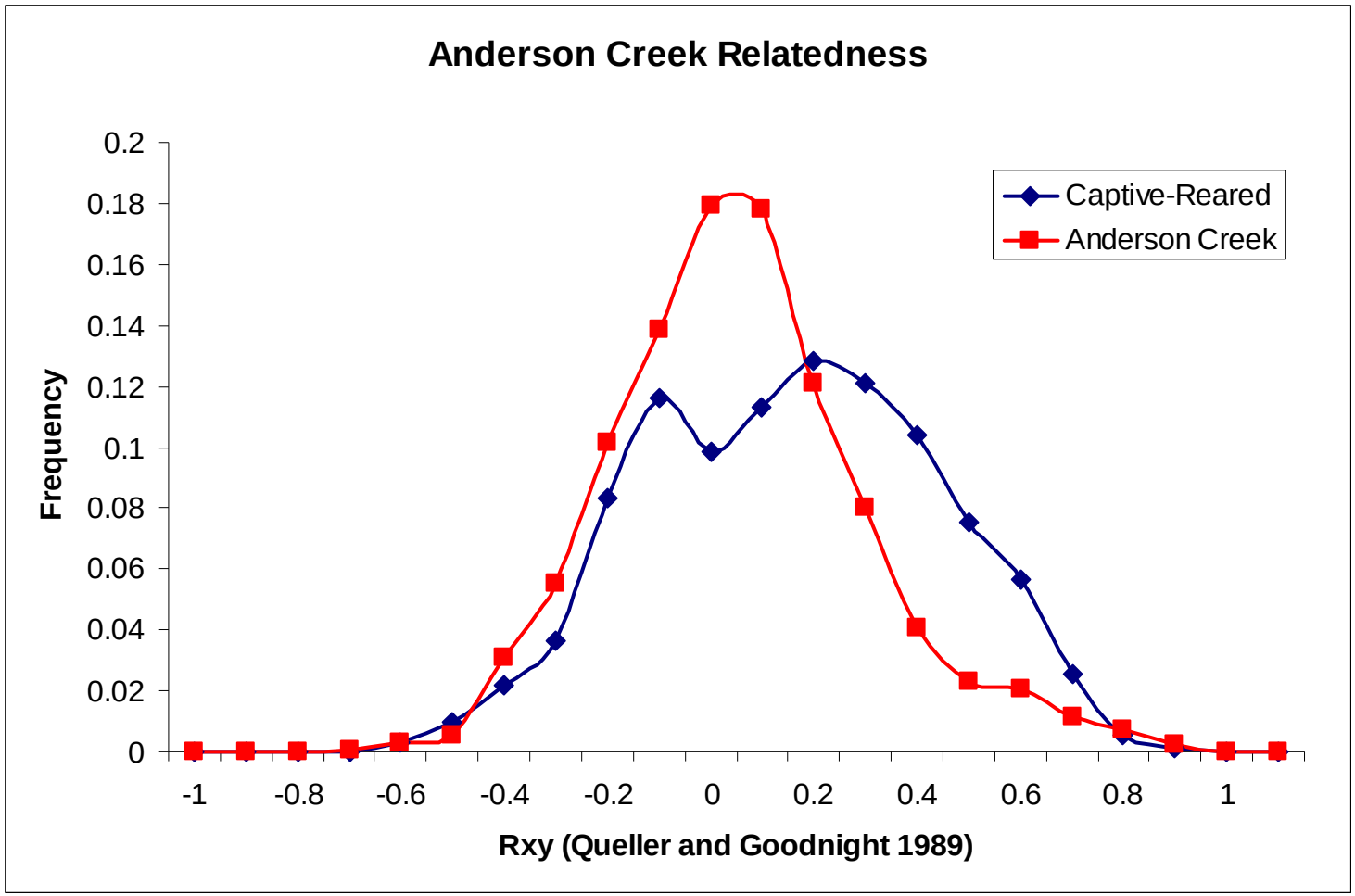

Figure 5. Distribution of pairwise relatedness values (Rxy) for juvenile bull trout collected in Anderson Creek and Captive-reared bull trout fry from 2007. We found a significant difference (Wilcoxon test $P<0.0001$) in the distribution of relatedness values between the two groups

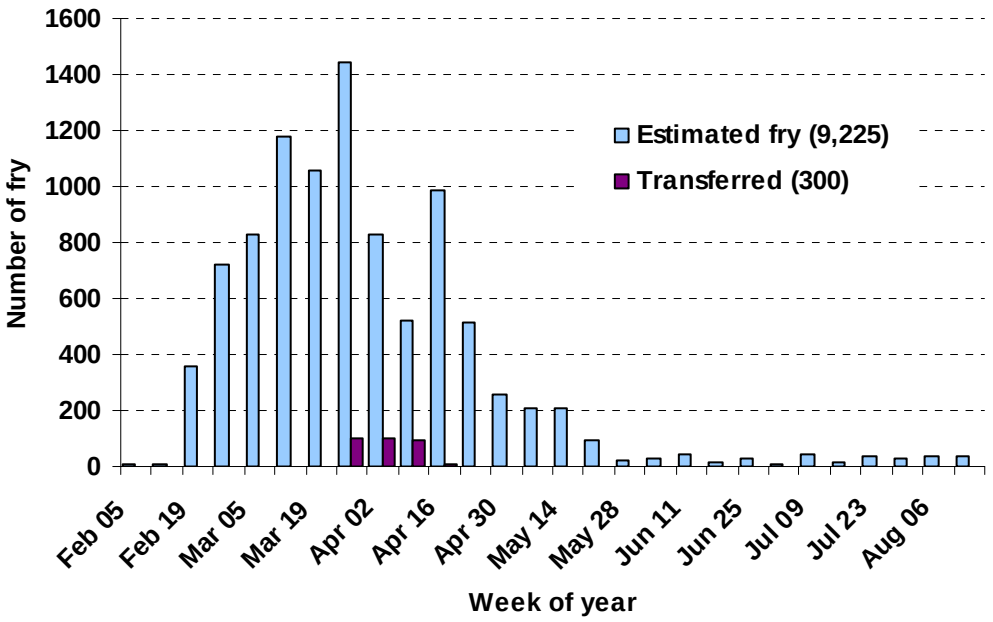

Figure 6. Estimated number and timing of bull trout fry moving downstream past the Anderson Creek screw trap located below the Highway 126 culvert and the number and timing of bull trout fry transferred to Leaburg for captive-rearing in 2007. Data provided by the Oregon Department of Fish and Wildlife.

Appendix 1. Bull trout PCR multiplex primer concentrations and annealing temperatures.

Multiplex Set 1 **T_A= 54˚C**

Locus Name	Dye	Final Concentration
Sfo18	6FAM	0.3µM
Sco212	VIC	1.0µM
Sco220	NED	3.3µM
Sco216	PET	4.0µM
Sco109	6FAM	6.6µM

Multiplex Set 2 **T_A= 59˚C**

Locus Name	Dye	Final Concentration
Sco202	6FAM	0.6µM
Sco102	PET	1.0µM
Sco215	PET	1.3µM
Sco200	VIC	2.0µM
Omm1128	VIC	2.0µM
Sco105	NED	1.3µM
Smm22	6FAM	4.6µM

Multiplex Set 3 **T_A=56˚C**

Locus Name	Dye	Final Concentration
Sco106	6FAM	1.0µM
Sco107	VIC	2.6µM
Omm1130	NED	5.3µM
Sco218	PET	3.3µM

T_A= Annealing temperature